Antibacterial Effects of Essential Oils on Oral Pathogens

1365

D.J. Birkenhauer • R. Peyyala • K.F. Novak • M.J. Novak

Center for Oral Health Research • University of Kentucky, Lexington, KY

Abstract

Essential oils are plant derived substances that have been shown to have antimicrobial activity. However, limited evidence of activity against oral bacteria is available. **Objective:** The present study was designed to ascertain if a composite formulation of three essential oils had antimicrobial activity against a panel of Gram positive and Gram negative oral bacteria. ORA MD is a commercially available composite of peppermint, spearmint, and almond oils and has been reported to be effective in the treatment of periodontal infection and inflammation. However, no objective studies are available to support these clinical observations. **Methods:** The antibacterial activity of the essential oils was assessed in triplicate against a panel of early, intermediate, and late plaque colonizers including *S. sanguis, S. oralis, S. gordonii, A. naeslundii, F. nucleatum, A. actinomycetemcomitans,* and *P. gingivalis* strains 381 and W83 with *S. aureus* as a non-oral control. A spectrophotometric assessment of inhibition of planktonic growth and a growth inhibition zone assay on agar plates using filter paper discs were used for each species and strain. **Results:** The composite of essential oils differentially inhibited the growth of all species and strains tested using either the spectrophotometric assay at 2µl essential oils/ml media or the plate assay at 1µl/mm of filter paper disc. The essential oils were more effective against the Gram negative species and strains than against the Gram positive species and least effective against *S. aureus*. **Conclusions:** The composite mixture of peppermint, spearmint, and almond oils has effective antibacterial activity against Gram positive and Gram negative oral bacteria although appears to be most effective against Gram negative species. This suggests that the beneficial clinical effects in reducing periodontal inflammation may be due to the antibacterial effects of the oils. Further studies are needed to elucidate the relative antibacterial activities of each oil independently.

Materials & Methods

Bacterial Strains and Cultures

Bacterial strains consisted of *Staphylococcus aureus, subspec. aureus* 25923, *Streptococcus sanguinis* 10556, *Streptococcus oralis*10558, *Streptococcus gordonii* 10557, *Aggregatibacter actinomycetemcomitans* JP2, *Fusobacterium nucleatum* 25586, *Actinomyces naeslundii* 49340, and *Porphyromonas gingivalis* 381 and W83.

Planktonic bacterial culture. Bacteria stocks were preserved at - 80°C, thawed to room temperature, and spread onto blood agar plates. Plates were maintained under anaerobic conditions (85% N_2, 5% CO_2 and 10% H_2) at 37°C. Each bacterial isolate was transferred into Bacto® Brain Heart Infusion (BHI), with added supplements hemin-5µg/ml and Menadione-1 µg/ml, and sub-cultured biweekly under the same anaerobic conditions.

Agar plate culture. Remel® CDC Formulation Blood Agar plates were spread with a 100 µL suspension of planktonic bacteria adjusted to 1x10^6 cells per mL.

Zone inhibition assay. Inhibition was assayed on agar plates prepared with the above concentration of cells. Plates were allowed to set for two hours at 4 degrees Celsius and after this time period 3 x 7mm discs of Whatman® filter paper #1 were coated with 7 µL of ORA MD® and placed into each of three distinct zones of the agar plate. After a 24 hour and 48 hour period at 37 degrees, Vernier® calipers were used to take 4 measurements of each zone of inhibition. A BioRad light table was used to aid in visualizing zones. This same protocol was used in anaerobic conditions in a controlled atmosphere chamber with a gas composition of 5% carbon dioxide, 10% Hydrogen, and 85% Nitrogen (Purity Plus, Lexington, Kentucky).

Planktonic inhibition assay. The effectiveness of ORA MD® is also assessed on the growth curve of a planktonic bacteria. A concentration of 2µL per mL of ORA MD was added to a starting bacterial suspension which was then measured every hour until the bacteria reached stationary growth phase. Results were compared to an untreated control culture.

Results

Figure 1: Gram (+) Panel Bacteria Growth Curves Treated versus Control

Figure 1 shows the growth curves of all Gram +ve bacteria from our sample panel. This graph displays the growth measured incrementally for seven hours by 600 nm light. Control bacteria cultures were untreated in BHI Media. Treated bacterial samples were tested in the same manner with testing running in parallel with control samples. Mean ± standard deviations of triplicate determinations are given. Treated samples were blanked using a standard curve of ORA MD® in BHI media .

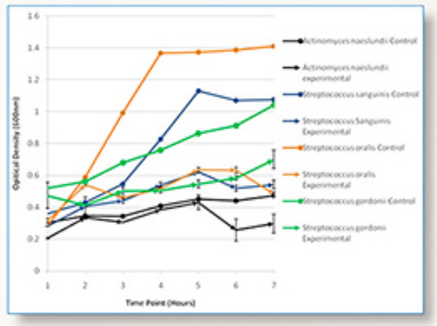


Figure 2: Gram (-) Panel Bacteria Growth Curves Treated versus Control

Figure 2 displays the growth curves of all Gram-Negative bacteria from our sample panel. This graph displays the growth measured incrementally by 600 nm light. Samples were run for varying amounts of time depending on their growth cycles. Control bacteria cultures were untreated in BHI Media. Treated and control bacterial samples were tested in triplicate and expressed as Mean ± standard deviations. Treated samples were blanked using a standard curve of ORA MD® in BHI media.

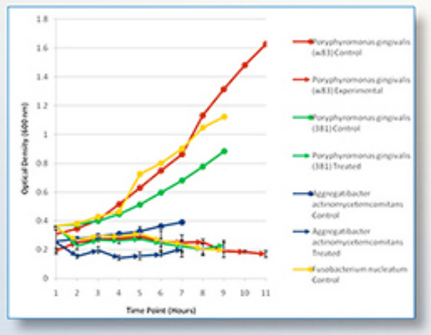


Figure 3: Gram Negative Panel Bacteria Comparison

Figure 3 shows trend line analysis of each treated and untreated Gram negative bacterial growth curve. Trend lines were created using exponential regression analysis for control bacteria and linear regression analysis for treated samples. The figure also shows the length of time that each treatment was run and determined to be effective for treated samples.

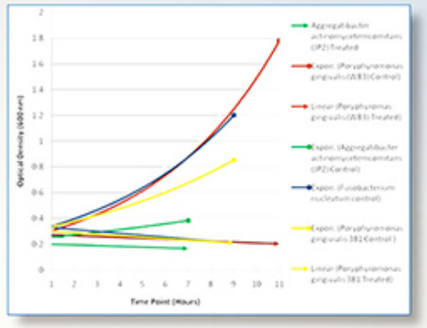
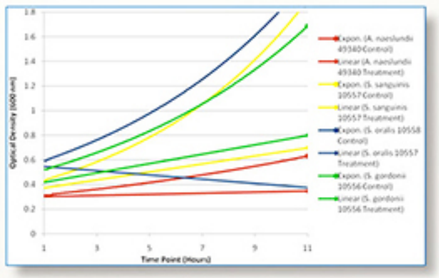


Figure 4: Gram Positive Panel Bacteria Comparison

Figure 4 shows trend line analysis of each treated and untreated Gram positive bacterial growth curve. Trend lines were created using exponential regression analysis for control bacteria and linear regression analysis for treated samples.



Conclusions

The composite mixture of peppermint, spearmint, and almond oils has effective antibacterial activity against Gram positive and Gram negative oral bacteria although appears to be most effective against Gram negative species. This suggests that the beneficial clinical effects in reducing periodontal inflammation may be due to the antibacterial effects of the oils. Further studies are needed to elucidate the relative antibacterial activities of each oil.

This is a proof of the abstract that will be available on the University of Kentucky website in March 2011. The final paper has been submitted for publication, and will be available soon thereafter.

July 1, 2010

Research Results for Ora MD®.

Daniel Birkenhauer

Rebecca Peyyala

Panel Bacteria

Bacteria	Strain	Gram Classification
P. gingivalis	381	Negative
A. naeslundii	49340	Positive
F. nucleatum	49256	Negative
A. actinomycetemcomitans	JP2	Negative
S. oralis	10556	Positive
S. Sanguinis		Positive
S. gordonii		Positive

Zone of Inhibition test.

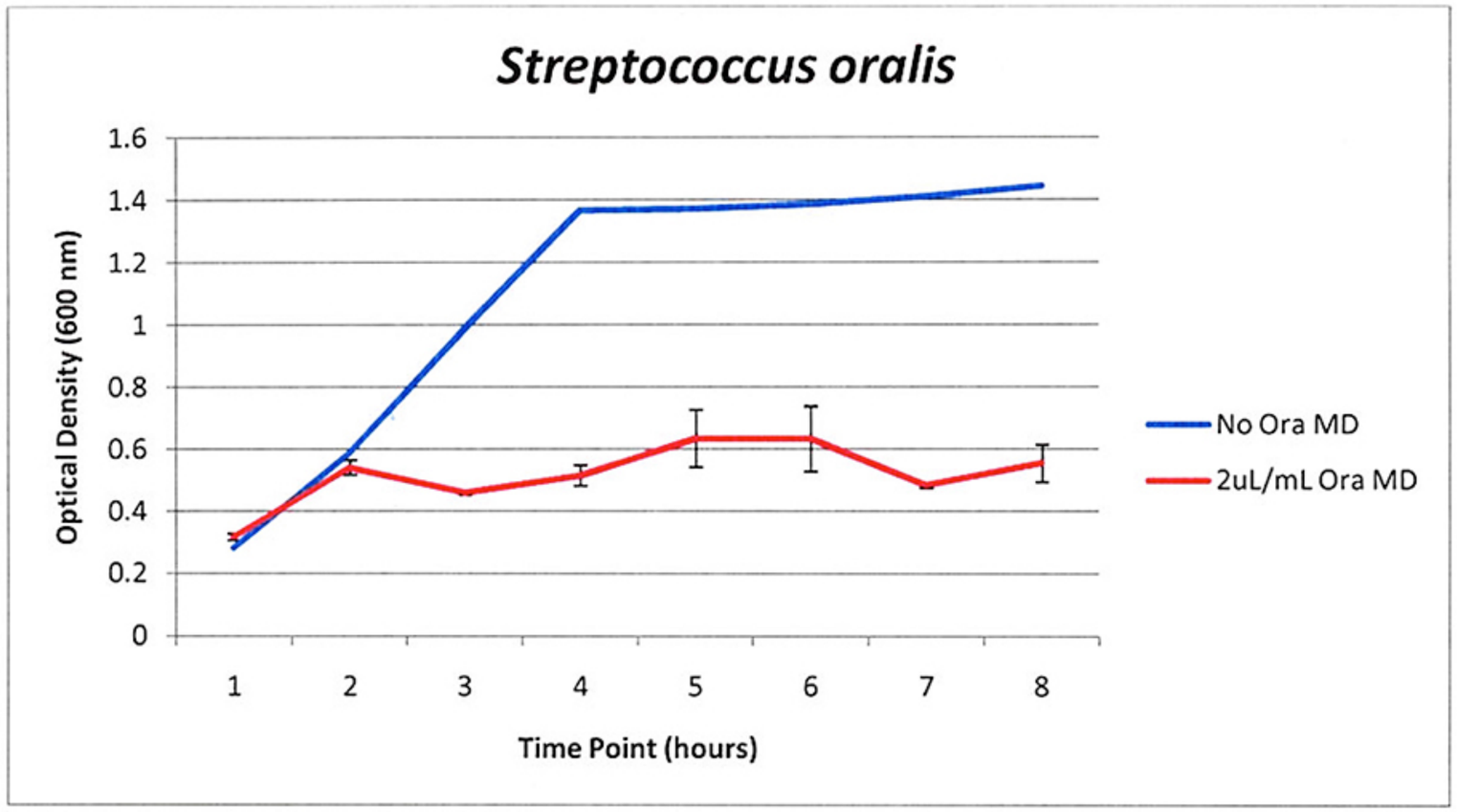
Completed with 7uL of Ora MD on a 7mm Watmann Filter Paper #1 Disc

Measured at 24 hours with calipers

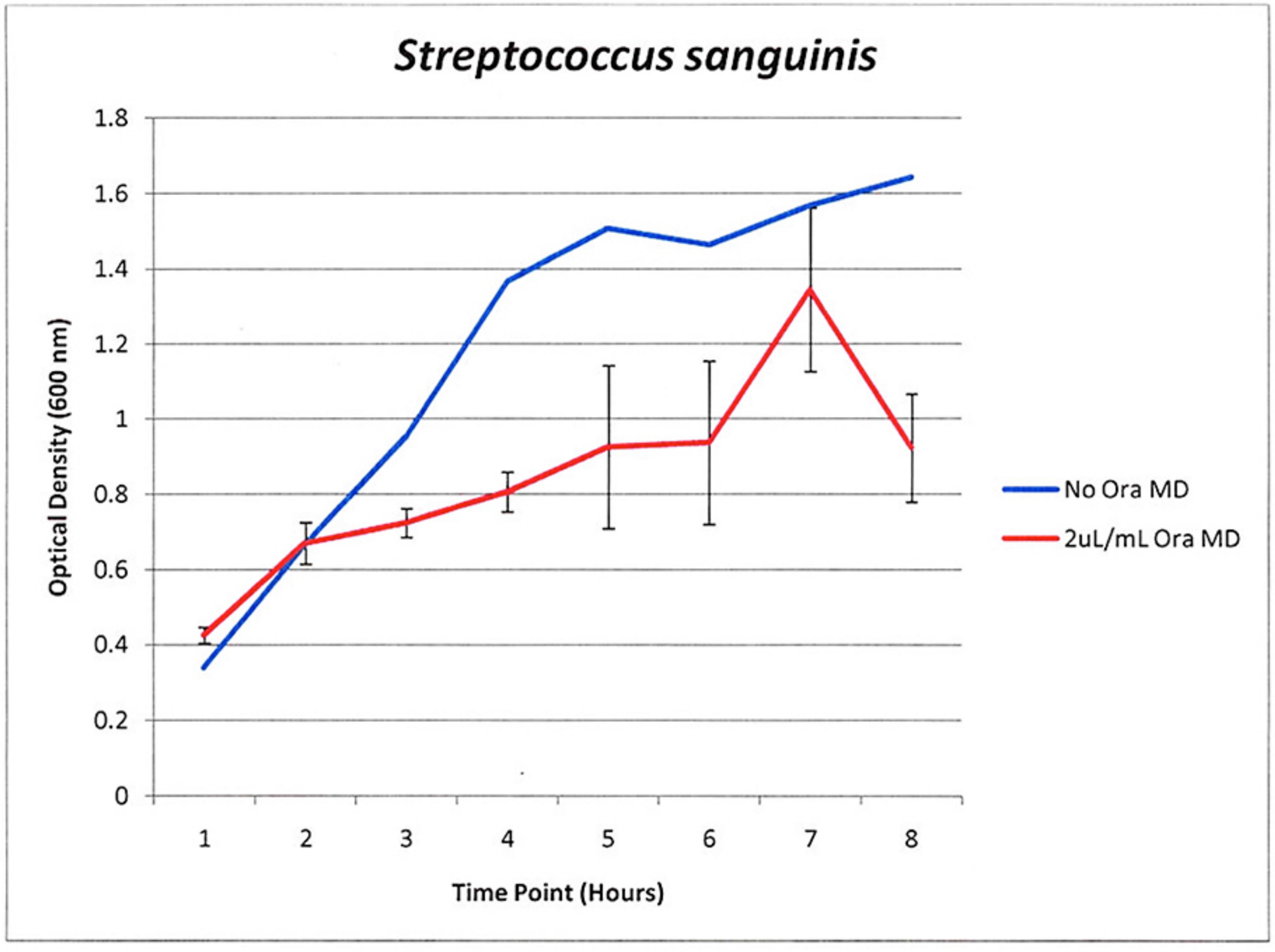
Each plate was covered with 100 uL of bacterial suspension at 0.1 OD and spread using a Fisher Spreader.

All controls showed negative for any inhibitory ability against any strain and were themselves sterile when placed on uninoculated agar plates.

Not conclusively inhibitory for A.N. aerobic, AA anaerobic/aerobic.

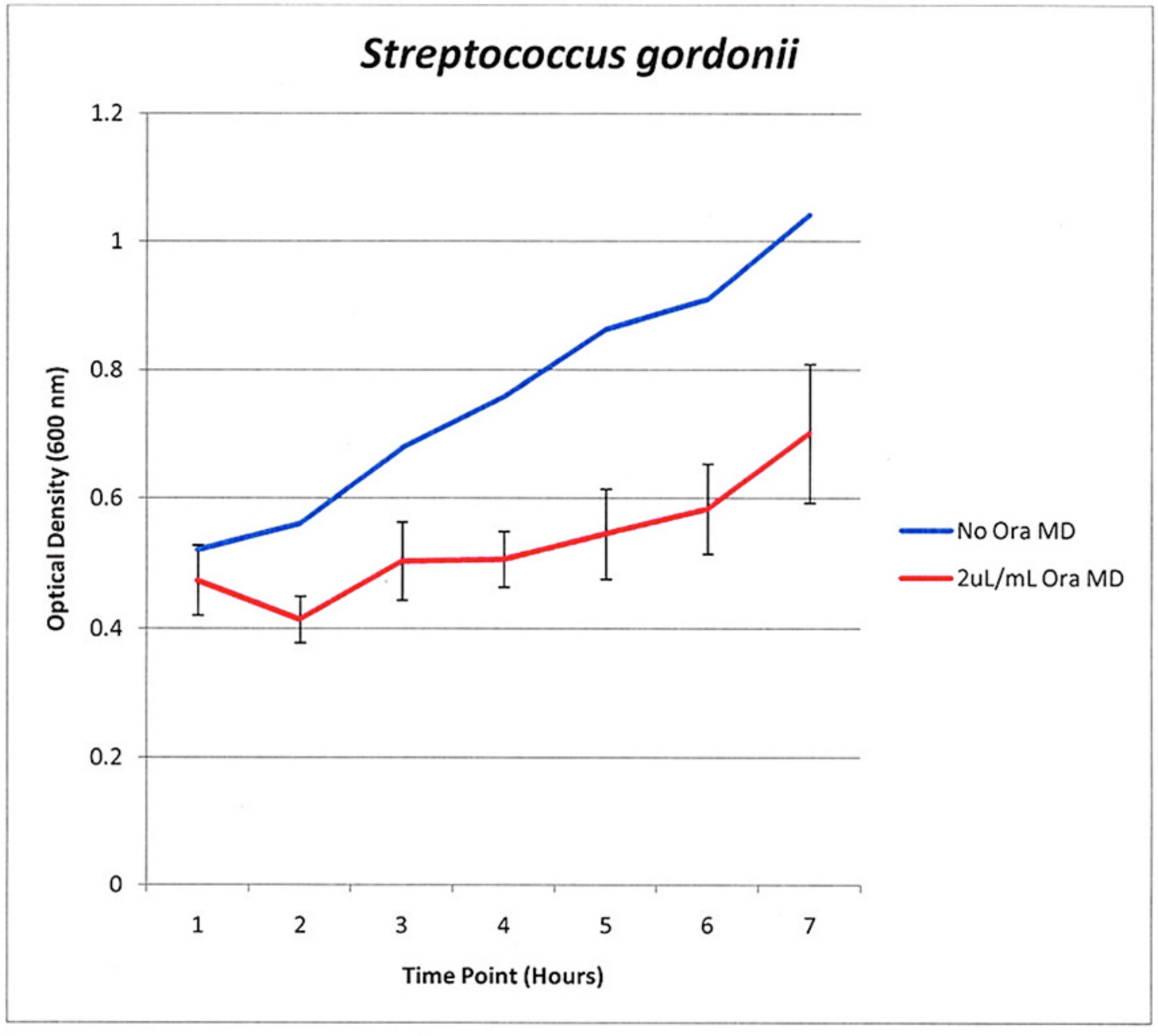


oralis		
control	Average	Std Dev
0.283	**0.317**	0.010614
0.59	**0.542**	0.023678
0.992	**0.461**	0.005888
1.367	**0.516333**	0.033767
1.372	**0.635333**	0.0925
1.385	**0.633333**	0.105282
1.41	**0.484**	0.007874
1.442	**0.555**	0.060117

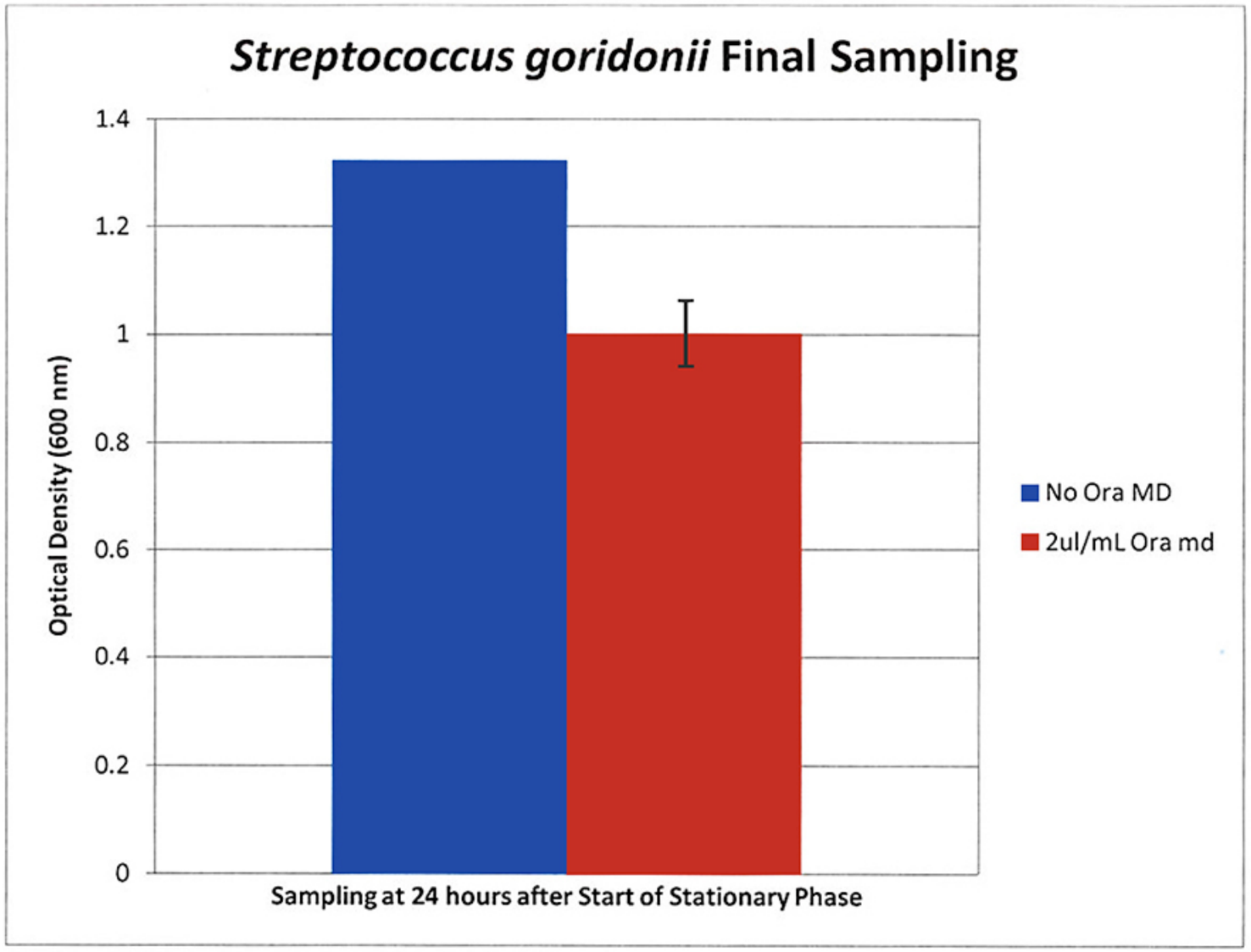


sanguinis

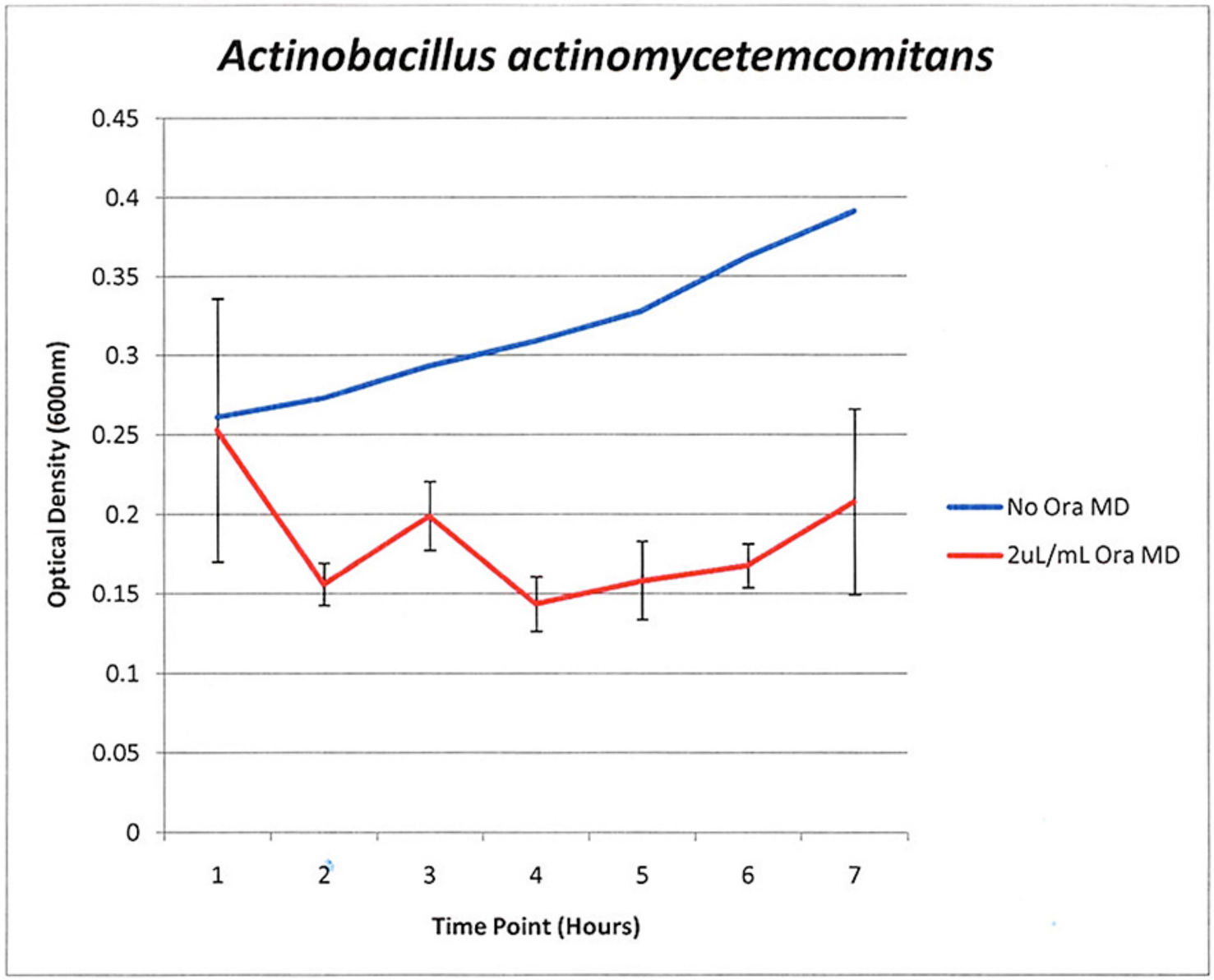
control	Average	Std Dev.
0.339	0.425	0.021924
0.666	0.67	0.054851
0.953	0.723333	0.036809
1.365	0.805333	0.053018
1.506	0.925	0.216196
1.464	0.936667	0.216995
1.567	1.343333	0.217282
1.644	0.922	0.143404



gordonii control	Average	Std Dev.
0.236		
0.521	0.474	0.053674
0.561	0.41375	0.035297
0.678	0.5035	0.059767
0.757	0.50625	0.042741
0.863	0.54525	0.069028
0.91	0.58375	0.069508
1.042	0.70075	0.107929

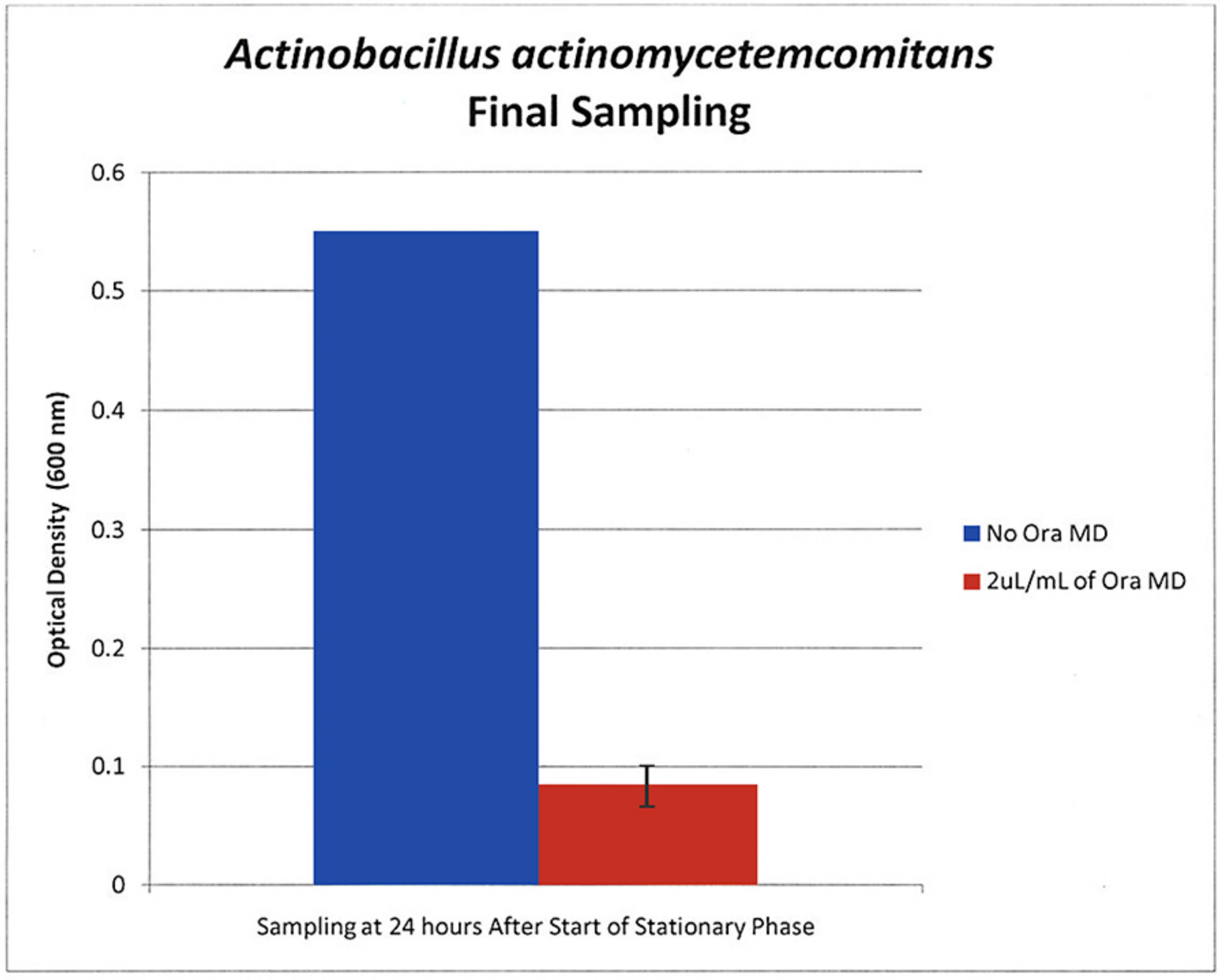


Control	Average	Std Dev
1.323	0.715	0.063

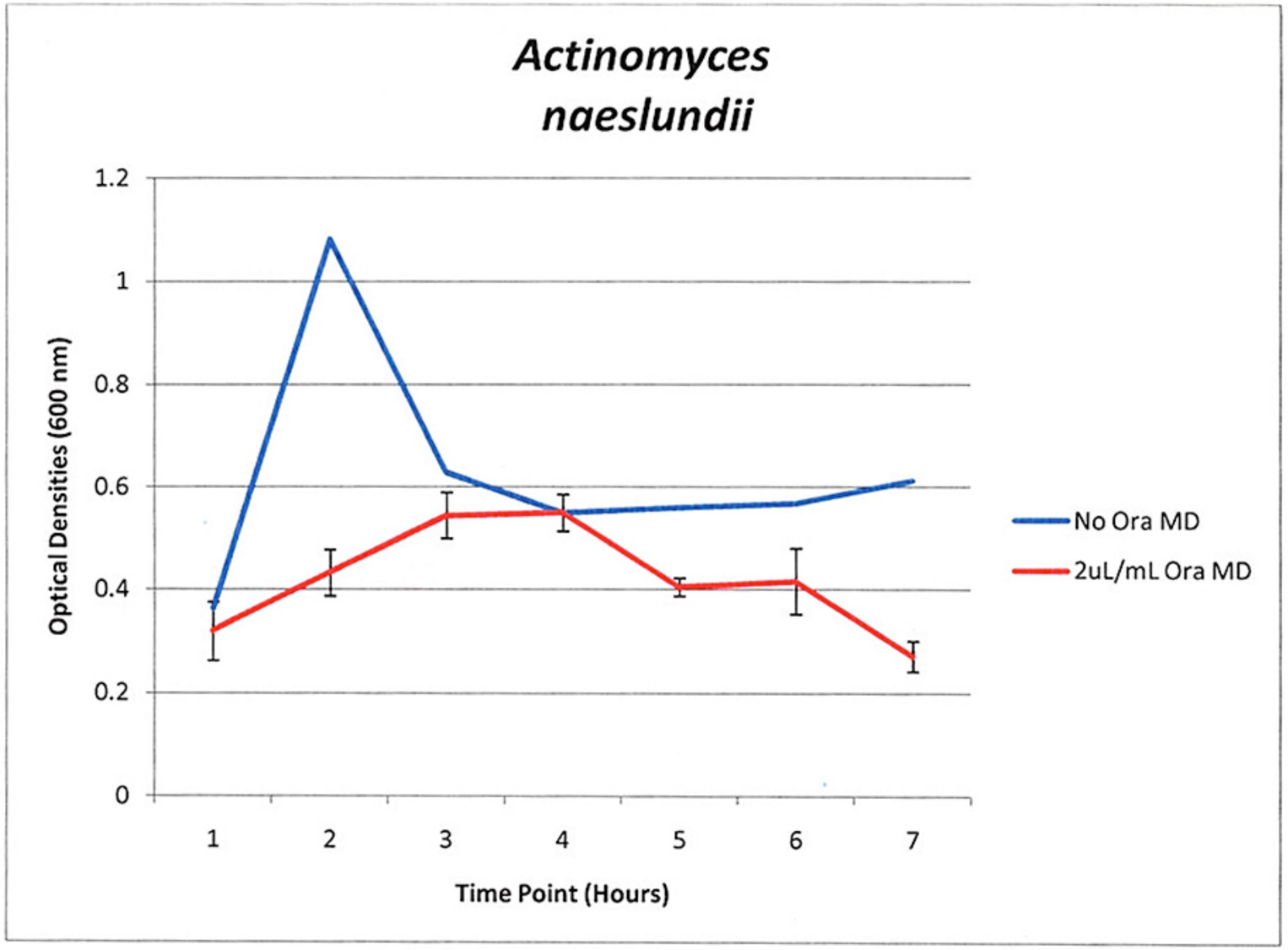


actinomycetemcomitans

control	Average	Std Dev
0.261	0.253	0.082877
0.273	0.156	0.013367
0.293	0.199	0.021772
0.309	0.143667	0.01725
0.328	0.158333	0.02473
0.362	0.167667	0.013816
0.391	0.207667	0.05822

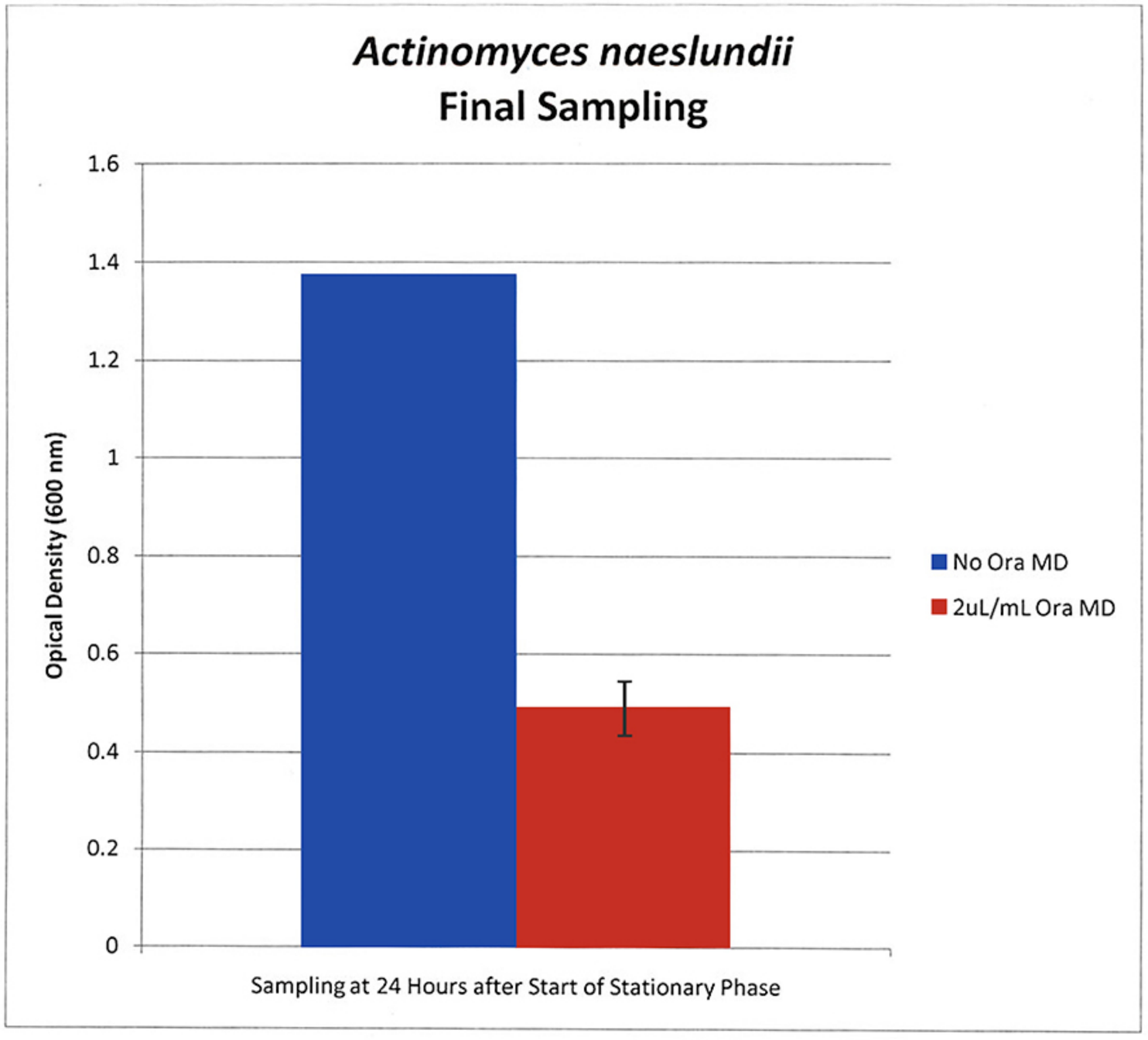


After 24 hours	Control	Average	Std Dev
	0.55	0.084667	0.01674



naeslundii

control	Average	Std Dev
0.362	0.319333	0.056038
1.082	0.433	0.045262
0.628	0.544333	0.044917
0.549	0.549667	0.03565
0.559	0.405667	0.017153
0.567	0.416333	0.064158
0.612	0.271667	0.029101



Control	Average	Std Dev
1.373	0.49	0.052428

Conclusions:

Based upon 2uL/mL of Ora MD in bacterial suspension Ora MD is either bacteriocidal or inhibitory towards all bacteria tested in our experimentation.